#4

Nucleotide and Amino Acid Sequences of Rat HICP

```
GACGCTTCTG ATCTCCAGAG GACCCTGGGG TGGGACAGGG GCCTTGGCAA GGCTGCAGCC      60

GCTGGGCAGT GGCTTGGAAT GGAGGTCTTT ATTACTGGGA ACTGAGGAGC TAAGAGGCTC     120

CTGTCAGCTT GTCCTAAAGT CTTAGCACTT GTGGTGGCTT GGGCTTCACA CACTGTCAGA     180

CACCTTCGTG GTGGCCTCCA CGGCCTCACC TTCAGGTTTG AAGCTGGCTC CACAAGGGAC     240

ACGGTGAC ATG AGG GGC AGC CCA CTG ATC CAT CTT CTG GCC ACT TCC TTC       290
        Met Arg Gly Ser Pro Leu Ile His Leu Leu Ala Thr Ser Phe
          1               5                  10

CTC TGC CTT CTC TCA ATG GTG TGT GCC CAG CTG TGC CGG ACA CCC TGT       338
Leu Cys Leu Leu Ser Met Val Cys Ala Gln Leu Cys Arg Thr Pro Cys
 15                  20                  25                  30

ACC TGT CCT TGG ACA CCA CCC CAG TGC CCA CAG GGG GTA CCC CTG GTG       386
Thr Cys Pro Trp Thr Pro Pro Gln Cys Pro Gln Gly Val Pro Leu Val
                 35                  40                  45

CTG GAT GGC TGT GGC TGC TGT AAA GTG TGT GCA CGG AGG CTG GGG GAG       434
Leu Asp Gly Cys Gly Cys Cys Lys Val Cys Ala Arg Arg Leu Gly Glu
             50                  55                  60

TCC TGC GAC CAC CTG CAT GTC TGC GAC CCC AGC CAG GGC CTG GTT TGT       482
Ser Cys Asp His Leu His Val Cys Asp Pro Ser Gln Gly Leu Val Cys
         65                  70                  75

CAG CCT GGG GCA GGC CCT GGC GGC CAT GGG GCT GTG TGT CTC TTG GAT       530
Gln Pro Gly Ala Gly Pro Gly Gly His Gly Ala Val Cys Leu Leu Asp
     80                  85                  90

GAG GAT GAC GGT AGC TGT GAG GTG AAT GGC CGC AGG TAC CTG GAT GGA       578
Glu Asp Asp Gly Ser Cys Glu Val Asn Gly Arg Arg Tyr Leu Asp Gly
 95                  100                 105                 110

GAG ACC TTT AAA CCC AAT TGC AGG GTC CTG TGC CGC TGT GAT GAC GGT       626
Glu Thr Phe Lys Pro Asn Cys Arg Val Leu Cys Arg Cys Asp Asp Gly
                115                 120                 125

GGC TTC ACC TGC CTG CCG CTG TGC AGT GAG GAT GTG CGG CTG CCC AGC       674
Gly Phe Thr Cys Leu Pro Leu Cys Ser Glu Asp Val Arg Leu Pro Ser
            130                 135                 140

TGG GAC TGC CCA CGC CCC AAG AGA ATA CAG GTG CCA GGA AAG TGC TGC       722
Trp Asp Cys Pro Arg Pro Lys Arg Ile Gln Val Pro Gly Lys Cys Cys
        145                 150                 155

CCC GAG TGG GTA TGT GAC CAG GGA GTG ACA CCG GCG ATC CAG CGC TCC       770
Pro Glu Trp Val Cys Asp Gln Gly Val Thr Pro Ala Ile Gln Arg Ser
    160                 165                 170

ACG GCG CAA GGA CAC CAA CTT TCT GCC CTT GTC ACT CCT GCC TCT GCT       818
Thr Ala Gln Gly His Gln Leu Ser Ala Leu Val Thr Pro Ala Ser Ala
175                 180                 185                 190

GAT GCT CCT TGT CCA AAT TGG AGC ACA GCC TGG GGC CCC TGC TCA ACC       866
```

FIGURE 1

Nucleotide Sequence Encoding Mature HICP and the Amino Acid Sequence of Mature HICP

```
CAG CTG TGC CGG ACA CCC TGT ACC TGT CCT TGG ACA CCA CCC CAG TGC          48
Gln Leu Cys Arg Thr Pro Cys Thr Cys Pro Trp Thr Pro Pro Gln Cys
  1               5                  10                  15

CCA CAG GGG GTA CCC CTG GTG CTG GAT GGC TGT GGC TGC TGT AAA GTG          96
Pro Gln Gly Val Pro Leu Val Leu Asp Gly Cys Gly Cys Cys Lys Val
            20                  25                  30

TGT GCA CGG AGG CTG GGG GAG TCC TGC GAC CAC CTG CAT GTC TGC GAC         144
Cys Ala Arg Arg Leu Gly Glu Ser Cys Asp His Leu His Val Cys Asp
        35                  40                  45

CCC AGC CAG GGC CTG GTT TGT CAG CCT GGG GCA GGC CCT GGC GGC CAT         192
Pro Ser Gln Gly Leu Val Cys Gln Pro Gly Ala Gly Pro Gly Gly His
    50                  55                  60

GGG GCT GTG TGT CTC TTG GAT GAG GAT GAC GGT AGC TGT GAG GTG AAT         240
Gly Ala Val Cys Leu Leu Asp Glu Asp Asp Gly Ser Cys Glu Val Asn
65                  70                  75                  80

GGC CGC AGG TAC CTG GAT GGA GAG ACC TTT AAA CCC AAT TGC AGG GTC         288
Gly Arg Arg Tyr Leu Asp Gly Glu Thr Phe Lys Pro Asn Cys Arg Val
                85                  90                  95

CTG TGC CGC TGT GAT GAC GGT GGC TTC ACC TGC CTG CCG CTG TGC AGT         336
Leu Cys Arg Cys Asp Asp Gly Gly Phe Thr Cys Leu Pro Leu Cys Ser
            100                 105                 110

GAG GAT GTG CGG CTG CCC AGC TGG GAC TGC CCA CGC CCC AAG AGA ATA         384
Glu Asp Val Arg Leu Pro Ser Trp Asp Cys Pro Arg Pro Lys Arg Ile
        115                 120                 125

CAG GTG CCA GGA AAG TGC TGC CCC GAG TGG GTA TGT GAC CAG GGA GTG         432
Gln Val Pro Gly Lys Cys Cys Pro Glu Trp Val Cys Asp Gln Gly Val
    130                 135                 140

ACA CCG GCG ATC CAG CGC TCC ACG GCG CAA GGA CAC CAA CTT TCT GCC         480
Thr Pro Ala Ile Gln Arg Ser Thr Ala Gln Gly His Gln Leu Ser Ala
145                 150                 155                 160

CTT GTC ACT CCT GCC TCT GCT GAT GCT CCT TGT CCA AAT TGG AGC ACA         528
Leu Val Thr Pro Ala Ser Ala Asp Ala Pro Cys Pro Asn Trp Ser Thr
                165                 170                 175

GCC TGG GGC CCC TGC TCA ACC ACC TGT GGG CTG GGC ATA GCC ACC CGA         576
Ala Trp Gly Pro Cys Ser Thr Thr Cys Gly Leu Gly Ile Ala Thr Arg
            180                 185                 190

GTG TCC AAC CAG AAC CGA TTC TGC CAA CTG GAG ATC CAA CGC CGC CTG         624
Val Ser Asn Gln Asn Arg Phe Cys Gln Leu Glu Ile Gln Arg Arg Leu
        195                 200                 205

TGT CTG CCC AGA CCC TGC CTG GCA GCC AGG AGC CAC AGC TCA TGG AAC         672
Cys Leu Pro Arg Pro Cys Leu Ala Ala Arg Ser His Ser Ser Trp Asn
    210                 215                 220
```

FIGURE 2

AGT GCT TTC
Ser Ala Phe
225

681

FIGURE 2 (Continued)

Alignment of the Modular Domains of HICP with the Modular Domains of Other CCN Family Members

MODULE I : IGFBP Domain

```
          28                  45 46                60 61                75 76                90 91      100
1 HICP    QLCRTPCT--CP-WTPPQC -PQGVPLVLDGCGCC KVCARRLGESCDHLH VCDPSQGLVCQPGAG PGGHGAVCLL
2 CTGF    QDCSAQCQ--CAAEAAPHC -PAGVSLVLDGCGCC RVCAKQLGELCTERD PCDPHKGLFCDFGSP ANRKIGVCTA
3 NOV     LRCPSRCPPKCPSIS-PTC AP-GVRSVLDGCSCC PVCARQRGESCSEMR PCDQSSGLYCDRSAD PNNQTGICMV
4 CYR61   -TCPAACH--CPLEA-PKC AP-GVGLVRDGCGCC KVCAKQLNEDCSKTQ PCDHTKGLECNFGAS STALKGICRA
            *   *   *       *              *****   **   *  *          **  *           *  *
```

MODULE II : vWFC Domain

```
          101                 120 121         135 136         150 151         165 166         180
1 HICP    DDGSCEVNGRRYLDGETFKP NCRVLCRCDDGGFTC LPLCSEDVRLPSWDC PRPKRIQVPGKCCPE WVC---------D-Q
2 CTGF    DGAPCVFGGSVYRSGESFQS SCKYQCTCLDGAVGC VPLCSMDVRLPSPDC PFPRRVKLPGKCCKE WVC---------DEP
3 NOV     EGDNCVFDGVIYRNGEKFEP NCQYFCTCRDGQIGC LPRCQLDVLLPGPDC PAPRKVAVPGECCEK WTCGS-------DEQ
4 CYR61   EGRPCEYNSRIYQNGESFQP NCKHQCTCIDGAVGC IPLCPQELSLPNLGC PNPRLVKVSGQCCEE WVCDEDSIKDSLDDQ
            *   *                 *   * *     *   *           *           * **
```

MODULE III : TSP1 Domain

```
          240         250                 265                 280                 298
1 HICP    PCPNWSTAWG PCSTTCGLGIATRVS NQNRFCQLEIQRRLC LPRPCLAARSHSSWNSAF-
2 CTGF    NCLVQTTEWS ACSKTCGMGISTRVT NDNTFCRLEKQSRLC MVRPCEADLEENIK-KGKK
3 NOV     NCIEQTTEWS ACSKSCGMGVSTRVT NRNRQCEMVKQTRLC IVRPCEQEPEEVTDKKGKK
4 CYR61   KCIVQTTSWS QCSKSCGTGISTRVT NDNPECRLVKETRIC EVRPCGQPVYSSLK-KGKK
                    *   *   *   *           *           *           *
```

FIGURE 3

Northern Blot Analysis of HICP Expression in Rat Aorta Smooth Muscle Cells

FIGURE 4

DNA Synthesis in Rat Aorta Smooth Muscle Cells

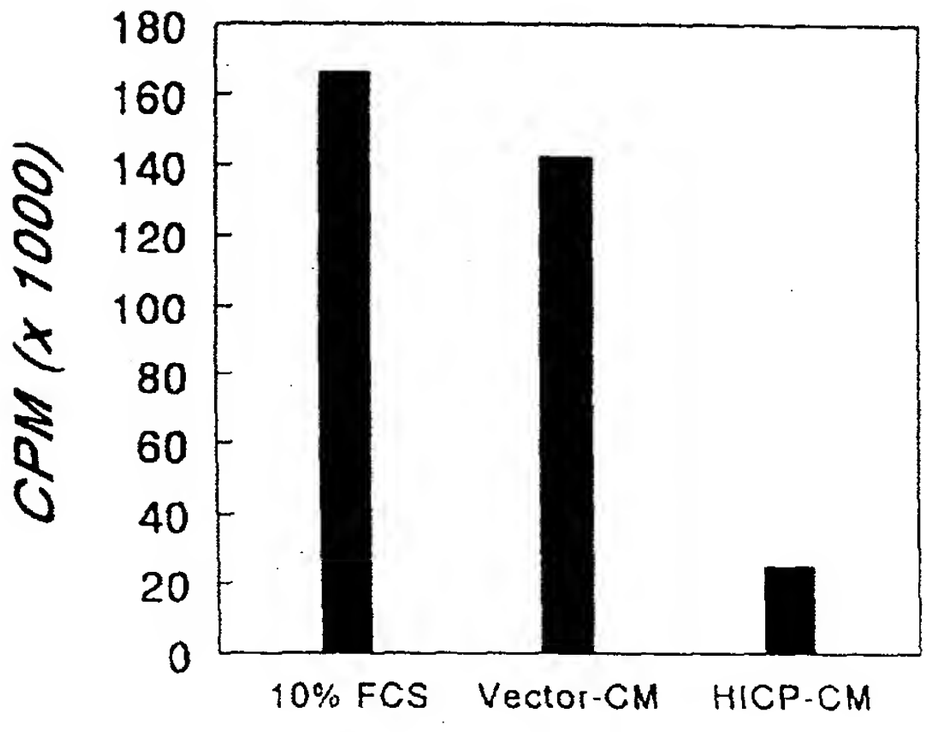

FIGURE 5